

# A Monoclonal Antibody Against the Nuclear Pore Complex Inhibits Nucleocytoplasmic Transport of Protein and RNA In Vivo

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**Abstract.** A monoclonal antibody that reacts with proteins in the nuclear pore complex of rat liver (Snow, C. M., A. Senior, and L. Gerace. 1987. *J. Cell Biol.* 104:1143–1156) has been shown to cross react with similar components in *Xenopus* oocytes, as determined by immunofluorescence microscopy and immunoblotting. We have microinjected the antibody into oocytes to study the possible role of these polypeptides in nucleocytoplasmic transport. The antibody inhibits import of a large nuclear protein, nucleoplamin, in a time- and concentration-dependent manner.

It also inhibits export of 5S ribosomal RNA and mature tRNA, but has no effect on transcription or intranuclear tRNA processing. The antibody does not affect the rate of diffusion into the nucleus of two small proteins, myoglobin and ovalbumin, indicating that antibody binding does not result in occlusion of the channel for diffusion. This suggests that inhibition of protein and RNA transport occurs by binding of the antibody at or near components of the pore that participate in mediated transport.

**M**OLECULAR traffic between the nuclear and cytoplasmic compartments of the eukaryotic cell takes place through pore complexes that perforate the nuclear envelope (for reviews see references 11, 14, and 23). The pore complex is a supramolecular protein assembly that forms an aqueous channel through the double-nuclear membrane at points where the inner and outer membranes are fused (23, 51). As seen by electron microscopy, the pore complex contains two eightfold symmetrical rings with outer diameters of 120 nm, one on each face of the envelope, which surround a central channel (24, 51). Radial spokes appear to project inward from the walls of the channel, where a central particle can often be found (51). Since transport appears to take place through the center of this channel (21), the central particle may contain material in the process of being transported.

The nuclear pore complex behaves as a molecular sieve that allows the passive diffusion of solutes at a rate inversely proportional to molecular size, up to a limit of 9–11 nm diam (2, 41–44). Thus, globular proteins of less than ~20 kD will readily diffuse across the envelope, while proteins of more than ~65 kD are effectively excluded (2). Because many nuclear proteins are larger than the limit for diffusion, or would diffuse too slowly across the envelope to account for their physiological rates of appearance in the nucleus (3, 20), transport of most nuclear proteins is thought to be achieved by a mediated mechanism.

Several nuclear proteins have been shown to contain short stretches of amino acids that confer a nuclear location on the protein (for examples see references 27, 31, 32, 34, 46, 53). This suggests that nuclear proteins might interact with one or several receptors for these sequences, most probably located in the pore complex. Export of RNA also appears to take place by a mediated mechanism that can be saturated, as suggested by work with transfer RNA (55). Therefore, receptors are also likely to exist for RNA and/or RNPs. Receptors appear to act in a vectorial fashion because proteins that are freely diffusible inside the nucleus (44) do not appear to be transported back to the cytoplasm.

The pore complex can transport very large particles, as demonstrated by injection of colloidal gold particles conjugated to a nuclear protein into the cytoplasm of *Xenopus* oocytes. Particles of more than 20 nm diam were found to pass through the central channel as readily as smaller particles (21). Transport of these gold particles was dependent upon their conjugation with a protein that contains a signal for nuclear location. The ability of the pore complex to transport such large particles suggests that the channel may be gated to open from 10 nm to >20 nm upon interaction with a protein containing a nuclear location signal. Evidence from in vivo and cell-free systems to study nuclear protein import indicate that transport is likely to be ATP dependent (38, 40, 45) and, in the absence of ATP, nuclear proteins will bind to the pore complex but will not be transported (39, 45).

Despite considerable interest in the ultrastructure and functions of the pore complex, relatively little is known about its molecular composition. An integral membrane glycoprotein of 190,000  $M_r$  (25) was identified biochemically

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by virtue of its relative abundance, and shown to be localized to the pore complex by immunocytochemistry. On the basis of its biochemical characteristics, this protein could plausibly serve to anchor the complex to the lipid bilayer (25). More recently, several groups have used monoclonal antibodies to identify less abundant protein components of the pore complex (9, 10, 28, 49). All of these antibodies recognize proteins that are *O*-glycosylated with *N*-acetyl glucosamine. The antibodies raised by Snow et al. identify several distinct epitopes on a group of eight pore complex polypeptides, in which the sugar residues contribute to the antibody binding site (30). These eight polypeptides may be important in nuclear transport for several reasons: there are estimated to be between two and eight copies of each polypeptide per pore complex, which probably contribute <15% of the pore complex mass (49), a figure more consistent with a functional role in transport than a purely structural role; they are located in the rings, the parts of the pore presented to the nucleoplasm and cytoplasm that might be expected to contain receptors and other parts of the translocation apparatus, and at least one of the polypeptides is asymmetrically disposed on only the nucleoplasmic face of the envelope, as one would expect for components involved in vectorial transport.

As a first step to study the possible functional roles of these pore complex proteins, we have examined the effects of the antibodies on nucleocytoplasmic transport *in vivo* in *Xenopus* oocytes. Here we present evidence that one antibody inhibits nucleocytoplasmic transport of protein and RNA, while having no discernable effect on diffusion through the pore. We conclude that transport may be inhibited by antibody binding at or near pore complex components that are involved in mediated nuclear transport.

## Materials and Methods

### Frogs and Oocytes

Mature *Xenopus laevis* were obtained from *Xenopus* I, (Ann Arbor, MI). They were kept at 20°C with a cycle of 15 h light, 9 h dark, and fed frog brittle (Nasco, Fort Atkinson, WI). Pieces of ovary were surgically removed after anaesthetizing the animal with 0.2% (wt/vol) 3-aminobenzoic acid ethyl ester in 50 mM Tris-HCl, pH 7.5. Ovary was washed briefly in OR-2 (82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, 5 mM Hepes-KOH, pH 7.8; Wallace et al. [52]) and oocytes were released from the ovary by digestion with 2 mg/ml collagenase (Type II; Sigma Chemical Co., St. Louis, MO) in calcium-free OR-2 for 2–3 h at room temperature. Mature stage 6 oocytes (17) were selected under the microscope and incubated overnight at 20°C in OR-2 before injection to select against those oocytes that die soon after isolation.

### Antibodies

RL1 (IgM) and RL7 (IgG1) are mouse monoclonal anti-pore complex antibodies described by Snow et al. (49). MSI (IgM) is a mouse monoclonal anti-ferritin, a generous gift of Dr. Mette Strand (Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD). The IgMs were purified from mouse ascites fluid by chromatography on Sephacryl S400 in 50 mM Tris-HCl, pH 8.8, 500 mM KCl, 0.02% (wt/vol) NaN<sub>3</sub>. The IgG was purified by protein A-Sepharose affinity chromatography as described by Ey et al. (19). All antibodies were concentrated on Amicon PM10 membranes (Amicon, Danvers, MA) and dialyzed against 50% (vol/vol) glycerol in PBS for storage at –20°C. Antibodies were conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's directions, to give a final antibody concentration of 4–5 mg/ml beads. For injection into oocytes, antibodies stored in glycerol were dialyzed extensively against D-PBS (Gibco

Laboratories, Grand Island, NY) and concentrated to 15 mg/ml on Centricon 30 (Amicon). Protein concentrations were determined by the method of Bradford (4) using mouse IgG as standard.

### Iodinated Proteins

Chicken egg white ovalbumin, sperm whale myoglobin, and *Staphylococcus aureus* protein A were obtained from Sigma Chemical Co. Nucleoplasmin was prepared from *X. laevis* eggs essentially as described by Dingwall et al. (16) except that the clarified extract was treated at 80°C for 10 min (35) before chromatography on DEAE cellulose and phenyl Sepharose. Nucleoplasmin, ovalbumin, and myoglobin were radioiodinated with [<sup>125</sup>I]Bolton-Hunter reagent (1) and dialyzed against 0.1 mM EDTA, 50 mM NaCl, 25 mM Hepes-NaOH, pH 7.5 to remove unreacted label. In the case of nucleoplasmin, unlabeled ovalbumin was added as a carrier before the dialysis step to give a final concentration of 2 mg/ml. The final concentration of <sup>125</sup>I was 1.5 × 10<sup>5</sup> cpm/μl, ~2 × 10<sup>6</sup> cpm/μg nucleoplasmin. More than 99% of the total <sup>125</sup>I was TCA insoluble. Protein A was iodinated by the chloramine-T procedure (26) to a final concentration of 2 × 10<sup>5</sup> cpm/μl, 10<sup>8</sup> cpm/μg.

### Protein Import Experiments

Oocytes, antibodies, and <sup>125</sup>I-labeled proteins were prepared for injection as described above. Oocytes were transferred to OR-2 containing 1% (wt/vol) Ficoll 400 (Sigma Chemical Co.) and injected in the vegetal hemisphere with 100 nl antibody or D-PBS using needles drawn from 0.008 in internal diameter precision bore glass tubing (Drummond Scientific, Broomall PA). After injection they were returned to OR-2 and incubated at 20°C for 2 h. They were then injected in the vegetal hemisphere with 25–50 nl <sup>125</sup>I-protein and incubated further at 20°C. At the appropriate times, oocytes were fixed with 20% (wt/vol) ice-cold TCA for 60 min (2), the nuclei were manually dissected from the oocytes and individual nuclei and cytoplasm were counted in a gamma counter. Transport into the nucleus was calculated as the percentage of the total cellular radioactivity in the nucleus for individual oocytes.

### RNA Export Experiments

Plasmid constructions were as previously described: pXbs1 contains a single copy of a somatic-type *Xenopus laevis* 5S ribosomal RNA gene (47), and pXlt<sup>me</sup> encodes a *Xenopus laevis* methionyl transfer RNA gene (6). DNAs were precipitated with ethanol, dissolved in D-PBS, and mixed with the appropriate antibody solution to give a final concentration of 0.2 mg/ml for each plasmid and 10 mg/ml antibody. Oocytes were incubated at 19°C throughout the experiments. For nuclear injections of antibody 25 nl of DNA-antibody solution was injected. The oocytes were incubated for 3 h, then newly synthesized RNA transcripts were labeled by injection of 25 nl α<sup>32</sup>P-GTP (20 mCi/ml, 600 Ci/mMol) (New England Nuclear, Boston, MA) followed by incubation for 2.5 h. For cytoplasmic injections, 50 nl of 15 mg/ml antibody solution was first injected into the vegetal hemisphere of the oocyte, followed 2 h later by injection of 25 nl of D-PBS containing 0.2 mg/ml pXbs and 0.2 mg/ml pXlt<sup>me</sup> into the nucleus. The oocytes were incubated for 2 h after injection of DNA, then RNA was labeled as described above. Nuclei and cytoplasm were manually dissected in 100 mM KCl, 20 mM NaCl, 3 mM MgCl<sub>2</sub>, 1 mM DTT, 10 mM Tris-HCl, pH 7.5, containing 2% (wt/vol) polyvinylpyrrolidone (37). For each condition nuclear and cytoplasmic fractions from 10 oocytes were pooled and homogenized in 0.5 ml 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.4, containing 1% (wt/vol) SDS and 100 μg/ml proteinase K (Boehringer Mannheim, Indianapolis, IN). RNA was purified by two phenol extractions followed by one chloroform extraction, and precipitated with ethanol. RNA was dissolved in a 95% (vol/vol) formamide-containing buffer and separated by denaturing electrophoresis on an 8% (wt/vol) polyacrylamide gel as described by Sakonju et al. (47).

### Immunofluorescence Microscopy

Small pieces of unfixed ovary were rapidly frozen in liquid nitrogen-cooled isopentane and embedded in O.C.T. compound (Miles Scientific, Naperville, IL). Sections of 5 μm were cut with a cryostat, the sections were fixed on microscope slides by immersion in ice-cold absolute methanol for 10 min, and returned to PBS without drying. Fixation of the ovary before freezing, or drying of sections resulted in loss of immunoreactivity. Sections were processed for indirect immunofluorescence microscopy using 17 μg/ml monoclonal antibody followed by rhodamine-conjugated goat anti-mouse

1. Abbreviations used in this paper: D-PBS, Dulbecco's formulation phosphate-buffered saline; WGA, wheat germ agglutinin.

IgG (Cappel Worthington Biochemicals, Malvern, PA) Photomicroscopy was performed using a Zeiss Photomicroscope III with a 25× objective and Tri-X pan film.

### SDS Gel Electrophoresis and Immunoblotting

Nuclei were isolated from oocytes that were fixed in ice-cold 20% (wt/vol) TCA for 15 min and solubilized in SDS gel sample buffer by sonication and boiling. The proteins were separated by SDS-PAGE as described by Maizel (36) and electroblotted onto nitrocellulose as described by Burnette (5) with the modifications of Gerace et al. (25). Blots were probed with monoclonal antibodies followed by rabbit anti-mouse IgG and <sup>125</sup>I-protein A, and processed for autoradiography as described by Snow et al. (49).

## Results

### Immunoreactivity of RL1 with the Nuclear Envelope of *Xenopus* Oocytes

A number of monoclonal antibodies that recognize the nuclear pore complex have been characterized in rat liver nuclear envelopes and cultured normal rat kidney cells (49). To use these antibodies to study nuclear transport by microinjection into *Xenopus* oocytes, we first established their cross-reactivity with *Xenopus*.

By indirect immunofluorescence microscopy all of these antibodies labeled the nuclear surface of the *Xenopus* A6 kidney cell line in a finely punctate pattern, like that seen with mammalian cells (data not shown). This indicates that the epitopes are all present in some *Xenopus* somatic cells, however, only RL1 was found to react strongly with *Xenopus* oocytes. On cryostat sections of ovary tissue RL1 stains the nuclear envelope exclusively (Fig. 1). It reacts with all stages of oocyte development. The complete nuclear envelope is most easily seen in early, pre-vitellogenic stages (Fig. 1, C and D). The appearance in mature oocytes (Fig. 1, A and B) is somewhat fragmented as a result of difficulty sectioning these large, unfixed cells. Interestingly, the nuclear envelopes of the follicle cells surrounding the oocyte are not stained by RL1. Antibody RL7, which reacts with a different epitope on a subset of the rat liver RL1 antigens (Snow, C., A. Senior, and L. Gerace, unpublished results; see Fig. 2 e) does not react with sections of any stage of oocyte (Fig. 1, E-H).

To identify the polypeptides recognized by RL1 in oocytes, nuclei were manually isolated and analyzed directly on immunoblots with RL1. The two major polypeptides that are labeled with RL1 have apparent molecular weights of 180,000 and 60,000 (Fig. 2, b and c). They are specifically labeled by RL1 but not by RL7 (Fig. 2 f) or MS1, a control antibody of the same class as RL1 (Fig. 2 h). These polypeptides are very similar in molecular weight to two major immunoreactive polypeptides found in rat liver nuclear envelopes (180,000 and 63,000 *M<sub>r</sub>*) (Fig. 2 a). When more nuclei are loaded onto the blot several more minor immunoreactive polypeptides can be identified (Fig. 2 d). No other major polypeptides are identified when whole oocytes are immunoadsorbed with RL1 and the adsorbed material is immunoblotted with the same antibody (data not shown). When considered together with the immunofluorescence data presented in Fig. 1, this provides good evidence that the antigens recognized by RL1 in oocytes are located in the nuclear envelope, and most probably are pore complex components of the *Xenopus* oocyte. This conclusion is supported by immuno-

gold labeling of the nuclear pore complexes with RL1 on whole-mount *Xenopus* oocyte nuclear envelopes (McDowell, A., and G. Griffiths, unpublished results).

### Inhibition of Nuclear Protein Import

To test the effect of RL1 on nuclear protein import into the nucleus we chose to use nucleoplasmin as a model protein. Nucleoplasmin is a large protein of *Xenopus* oocyte nuclei that contains nuclear location signals that allow its accumulation in the nucleus, apparently by a mediated transport mechanism (14–16). When <sup>125</sup>I-labeled nucleoplasmin is injected into the cytoplasm of oocytes it rapidly accumulates in the nucleus (16).

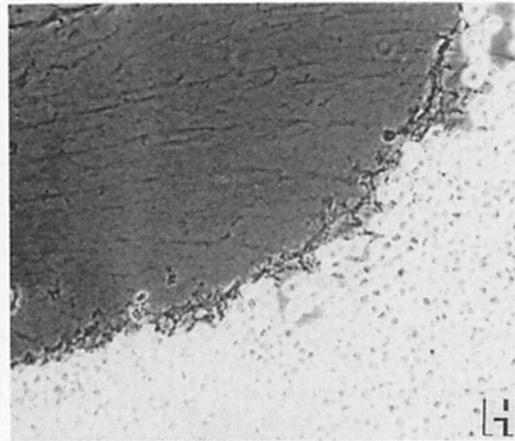
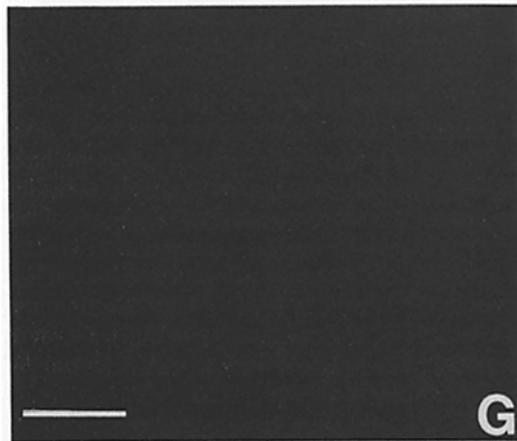
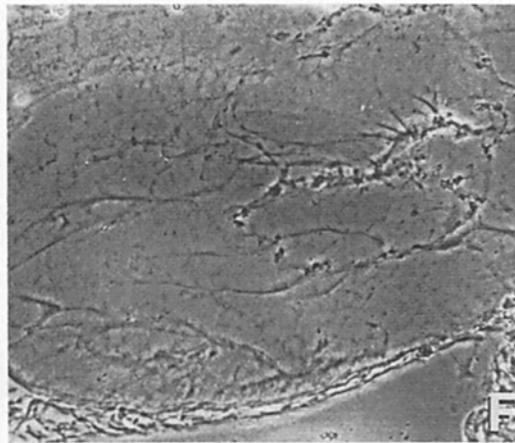
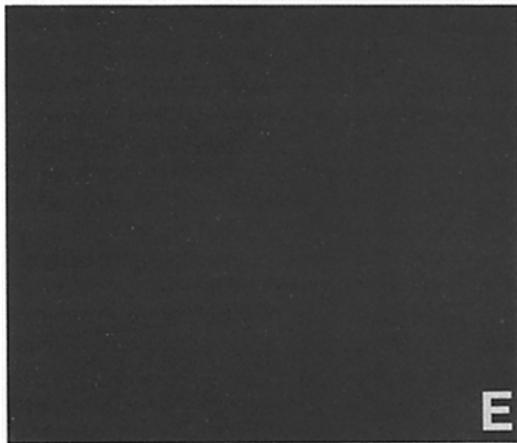
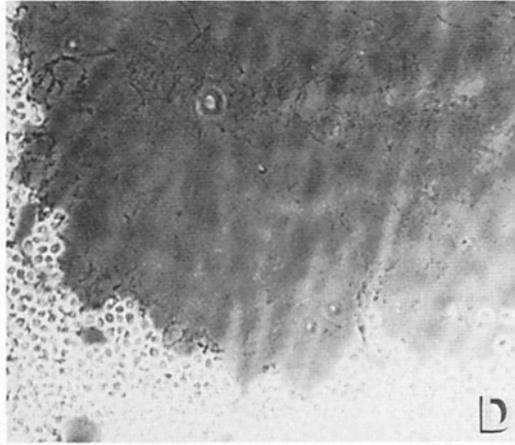
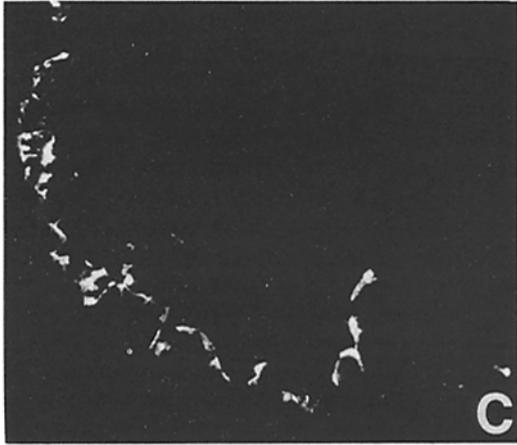
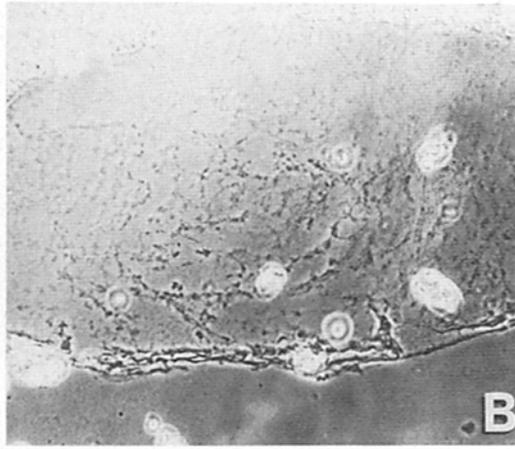
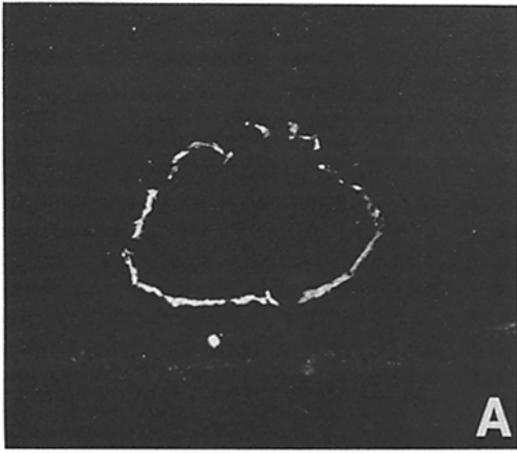
Oocytes were first injected with antibody to give a final cytoplasmic concentration of 1.0–1.5 mg/ml. They were then incubated at room temperature for 2 h to allow binding of the antibody before being injected with <sup>125</sup>I-nucleoplasmin. By SDS-PAGE and immunoblotting both nucleoplasmin and antibodies are stable in the oocyte for at least 24 h (data not shown). Oocytes injected with RL1 survive incubation at room temperature for more than 20 h. Over the course of 3 h oocytes were analyzed for the distribution of radiolabeled nucleoplasmin between nucleus and cytoplasm (Fig. 3). RL1 dramatically inhibits the accumulation of nucleoplasmin in the nucleus when compared to accumulation in control oocytes injected with buffer (Fig. 3 A). Neither RL7 (Fig. 3 B) nor MS1 (Fig. 3 C) significantly inhibits nucleoplasmin import in similar experiments. (The absolute amount of transport varies with the batch of oocytes used and the preparation of radiolabeled nucleoplasmin, therefore, all experiments are compared with transport in oocytes that were injected with buffer alone.) After a 2-h incubation, the mean inhibition by RL1 with respect to the D-PBS control was 55% for three separate experiments (Table I). This is probably a conservative estimate because the percentage of <sup>125</sup>I in the nucleus at time zero was always greater than zero. This may be due in part to rapid transport of nucleoplasmin into the nucleus (the approximate time between injection and fixation was ~2 min), to some freely diffusible <sup>125</sup>I in the nucleoplasmin preparation, or contamination of the nuclei with a small amount of adherent cytoplasm. In these experiments the mean value at time zero was 6.4% (30 measurements), thus the extent of inhibition is ~70%.

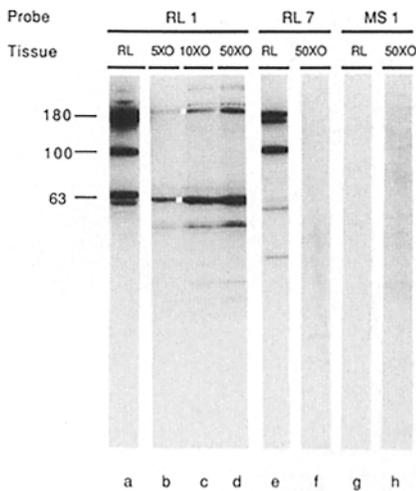
The extent of inhibition of transport at 2 h is dependent upon the concentration of injected antibody, with 50% inhibition at ~10 mg/ml in the needle (Fig. 4), which results in a final concentration of ~1 mg/ml in the oocyte cytoplasm, assuming that the volume of an oocyte is 1  $\mu$ l.

### Inhibition of RNA Export

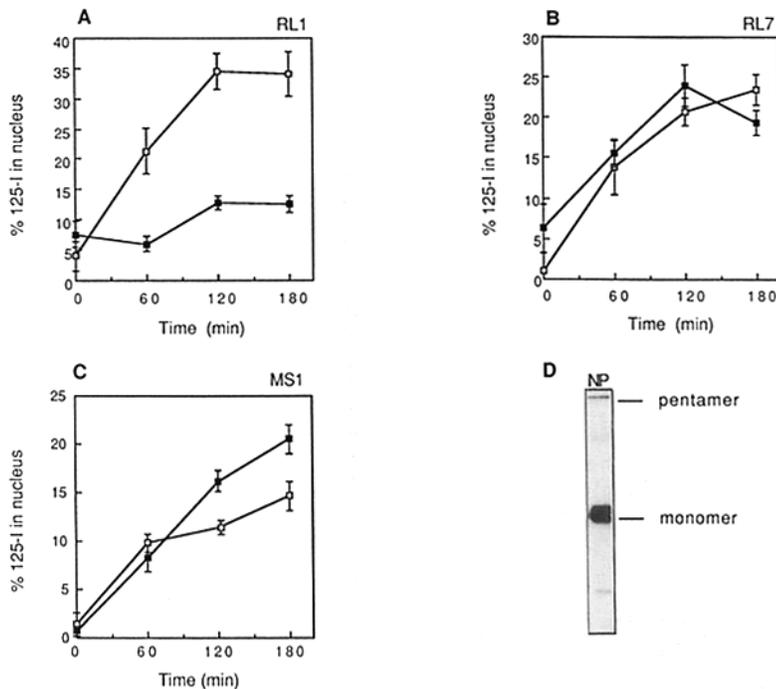
We extended these studies to consider export of RNA from the nucleus. To achieve a strong and specific signal we have used 5S RNA and tRNA<sup>met</sup> expressed from cloned genes injected into the nucleus. In these experiments a mixture of the two genes was injected into the nucleus along with RL1. The oocytes were incubated for 3 h, then newly synthesized RNA was labeled with  $\alpha$ -<sup>32</sup>P-GTP by injection of the label into the cytoplasm. After 2.5 h, the nuclei and cytoplasm were manually dissected, RNA was extracted and analyzed by acrylamide gel electrophoresis.

RL1 injected into the nucleus results in inhibition of export





**Figure 2.** Immunoblotting of *Xenopus* oocyte nuclei with RL1. *Xenopus* oocyte nuclei were manually dissected, subjected to SDS-PAGE, immunoblotted, and probed with RL1, RL7, or MS1. (Lanes a, e, and g) Salt-washed rat liver nuclear envelopes probed with RL1, RL7, and MS1, respectively. (Lanes b, c, and d) 5, 10, and 50 oocyte nuclei, respectively, probed with RL1. (Lanes f and h) 50 oocyte nuclei probed with RL7 and MS1, respectively. The markers to the left indicate the positions and relative molecular masses (kD) of three rat liver pore complex antigens. The white spots on lane b indicate the two major immunoreactive species in the oocyte.



**Figure 3.** Inhibition of nucleoplasm import by RL1. Stage 6 oocytes were injected in the vegetal hemisphere of the cytoplasm with antibodies (A–C, solid symbols) or with buffer alone (A–C, open symbols), incubated for 2 h then injected with  $^{125}\text{I}$ -labeled nucleoplasm. At the indicated times oocytes were fixed with TCA and dissected into nuclei and cytoplasm.  $^{125}\text{I}$  in the two fractions was determined by counting in a gamma counter. RL1 (A), RL7 (B), MS1 (C). The points are means of five oocytes, error bars indicate the SEMs. An autoradiogram of the iodinated nucleoplasm preparation after SDS-gel electrophoresis is shown in D.

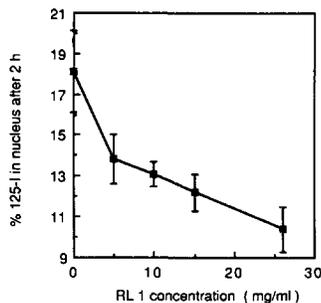
**Figure 1.** Immunofluorescence staining of *Xenopus* oocytes with RL1. 5- $\mu\text{m}$  cryostat sections of *Xenopus* ovary were fixed in ice-cold 100% methanol and labeled with RL1 (A–D) or RL7 (E–H) followed by rhodamine-conjugated rabbit anti-mouse. RL1 stains the nuclear envelope of all stages of oocyte, RL7 is negative for all stages. (A, B, E and F) Previtellogenic oocytes; (C, D, G, and H) mature oocytes. (A, C, E, and G) immunofluorescence. (B, D, F, and H) phase contrast. Bar, 50  $\mu\text{m}$ .

**Table I. Inhibition of Nucleoplasm Import by RL1**

	RL1	D-PBS	Inhibition by RL1
			%
% $^{125}\text{I}$ in nucleus after 2 h	11.9 $\pm$ 0.4 (3)	26.2 $\pm$ 3.4 (3)	55.6

Assays were performed as described in the legend to Fig. 3. The numbers shown are means  $\pm$  SEM for three experiments.

of 5S RNA and mature tRNA<sup>met</sup> when compared to control oocytes that were injected with D-PBS (Fig. 5). The control antibody RL7 has no effect on export of either RNA. RL1 does not inhibit transcription of either RNA species or the processing of tRNA to the mature form (tRNA precursors can be seen upon longer exposure). This indicates that injection of the antibody does not result in gross functional abnormalities in the nucleus. RL1 also inhibits RNA export when injected into the cytoplasm (Fig. 5 g–l). This inhibition is generally less marked than when the antibody is injected into the nucleus. Quantitation of inhibition of 5S and tRNA<sup>met</sup> export by RL1 is summarized in Table II. 5S RNA export was inhibited by 70% and tRNA<sup>met</sup> export was inhibited by 46% when RL1 was injected into the nucleus. RL7, and other anti-pore complex antibodies, had no significant effect on export. When injected into the cytoplasm, RL1 inhibited ex-



**Figure 4.** Titration of RL1 in the inhibition assay. Oocytes were injected with RL1 in D-PBS at concentrations in the needle as indicated. They were then incubated for 2 h before injection of <sup>125</sup>I-nucleoplasmin. Transport into the nucleus was assayed after 2 h as described in the legend to Fig. 3. The points are means of ten oocytes, error bars indicate the SEMs.

port of 5S RNA by 71%, but had only a slight inhibitory effect on tRNA<sup>met</sup> export. Inhibition of RNA export was more pronounced when export was saturated (55) by injecting larger amounts of plasmids containing multiple copies of the RNA genes (data not shown).

### Effect of RL1 on Diffusion Through the Pore

One obvious mechanism that could account for the inhibition of nuclear transport described above is wholesale occlusion of the aqueous channel of the pore complex as a result of antibody binding. To test the possibility that RL1 binding blocks the 10-nm channel for diffusion we analyzed the rate of diffusion of small proteins from cytoplasm to nucleus.

These experiments were performed exactly as with nucleoplasmin, using <sup>125</sup>I-labeled myoglobin (18,000 *M<sub>r</sub>*) and ovalbumin (43,000 *M<sub>r</sub>*). Myoglobin is much smaller than the limit for diffusion and equilibrates rapidly between the

**Table II.** Inhibition of RNA Export by RL1

	Injected into nucleus		Injected into cytoplasm	
	RL1	RL7	RL1	RL7
5S RNA	30 ± 6 (3)	113	29	99
tRNA <sup>met</sup>	54 ± 11 (3)	101	91	98

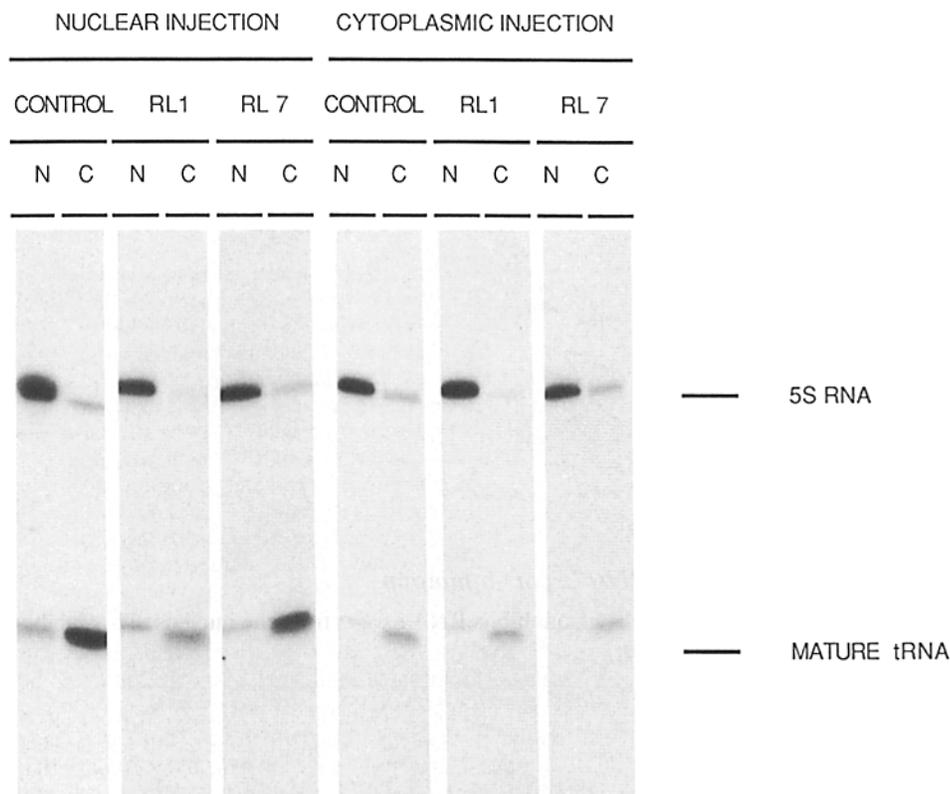
Assays were performed as described in the legend to Fig. 5. The radioactive gel bands were excised and counted in a scintillation counter and the percentage of each RNA exported was calculated with respect to the export seen with oocytes injected with D-PBS only. The numbers in the first column are means ± SEM for three experiments.

nucleus and cytoplasm (41). Previous injection of RL1 into the cytoplasm has no effect on the rate or extent of diffusion of this molecule (Fig. 6 *A*). Ovalbumin is closer to the size limit for exclusion from the nucleus, and so diffuses very slowly from cytoplasm to nucleus. Again, no significant difference in the rate of diffusion was observed over 15 h (Fig. 6 *B*). This data shows that antibody binding does not occlude the channel for passive diffusion, and the lack of effect on the rate of diffusion suggests that the overall diameter of the diffusion channel is not significantly altered by antibody binding.

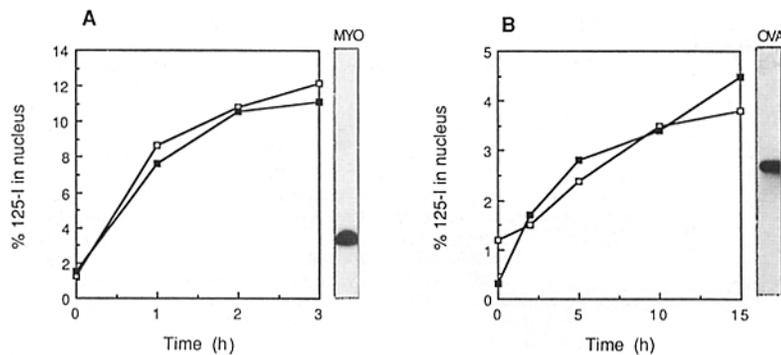
### Discussion

#### Cross-Reaction of RL Antibodies With *Xenopus* Oocytes

We have shown that RL1, a monoclonal antibody specific for the pore complex of rat liver nuclear envelopes, also labels



**Figure 5.** Inhibition of RNA export by RL1. Oocytes were injected either in the nucleus with a mixture of antibody and plasmids encoding 5S ribosomal RNA and tRNA<sup>met</sup> (nuclear injection), or in the vegetal hemisphere of the cytoplasm with antibody then in the nucleus with the plasmid DNAs (cytoplasmic injection). Newly transcribed RNA was labeled by injecting  $\alpha^{32}$ P-GTP. The nuclei (N) and cytoplasms (C) were dissected, the RNA was extracted and separated on a denaturing polyacrylamide gel. The gel was dried and subjected to autoradiography. (Control) Oocytes injected with D-PBS. (RL1) Oocytes injected with RL1. (RL7) Oocytes injected with RL7. Each lane represents two oocyte equivalents from a pool of 10 oocytes.



**Figure 6.** The effect of RL1 on diffusion of small proteins into the nucleus. The experiment was carried out as described in the legend to Fig. 3, but the oocytes were injected with  $^{125}\text{I}$ -labeled myoglobin (A) or  $^{125}\text{I}$ -labeled ovalbumin (B). Open symbols are oocytes injected with buffer alone, closed symbols are oocytes injected with RL1. The points are means of five oocytes. The panels show autoradiograms of the labeled proteins after SDS-gel electrophoresis.

the nuclear envelope of *Xenopus* oocytes and somatic cells. In rat liver the antibody reacts with a group of nuclear pore complex glycoproteins that share an epitope containing *N*-acetyl glucosamine linked to serine (30, 49). At least two polypeptides with similar apparent molecular weights to the rat liver polypeptides are recognized in *Xenopus* oocytes, suggesting that these are pore complex polypeptides bearing the same modification. This possibility is supported by the observation that the antibody stains *Xenopus* somatic cells in culture in a way typical of pore complex staining in other cell types, and by immunoelectron microscopy on whole-mount oocyte nuclear envelopes (McDowell, A., and G. Griffiths, unpublished results).

Among several antibodies tested, only RL1 reacted strongly with proteins in *Xenopus* oocytes. Since all of the antibodies label the pore complex of *Xenopus* A6 cells, this suggests that there is cell-specific expression of the epitopes within this species. This is supported further by the finding that the follicle cells surrounding the oocyte do not stain with RL1. This differential expression could be due to different extents of glycosylation of the polypeptides, or to expression of different protein isotypes in various cell types analogous to the cell-specific expression of another class of nuclear envelope proteins, the lamins (33, 50), as well as numerous other cellular proteins. This provides circumstantial evidence that the structure of the pore complex is modulated in different cell types, perhaps reflecting regulation of the functional properties of the pore complex in different cells.

### Inhibition of Nuclear Transport

Nucleoplasmin is an excellent model to study mediated protein import into the nucleus because it is very well-characterized with respect to its tertiary structure, stability, and ability to be transported into the nucleus after radioiodination (16). A COOH-terminal domain of  $\sim 5$  kD contains the information for nuclear location, since cleavage of this domain with proteases produces a transport-incompetent fragment. The COOH terminus contains several putative nuclear location signals, two similar in sequence to that of the SV-40 virus large T antigen (15, 31, 34). Large T antigen-like signal sequences have been found in several nuclear proteins (for examples see references 46, 53), suggesting that the transport apparatus is shared. It is, therefore, likely that RL1 will inhibit import of many nuclear proteins.

RL1 inhibits import of nucleoplasmin in a time- and concentration-dependent manner. Transport is unaffected by other antibodies that do not react with oocyte proteins, including other antibodies that recognize different epitopes on

the rat liver glycoproteins and a control IgM antibody. This suggests strongly that the antibody inhibits transport by binding to the pore complex of the oocyte, and confirms the morphological observations of Feldherr and colleagues that transport of nucleoplasmin (21) and RNA (18) takes place through the pore. Despite the likelihood that each pore complex binds several antibody molecules, RL1 does not prevent the passive diffusion of myoglobin or ovalbumin into the nucleus. Since the rate of diffusion is proportional to the diameter of the pore, the lack of effect of RL1 suggests that antibody binding does not significantly alter the 10-nm-diam channel for diffusion. From immunoelectron microscopy on rat liver nuclear envelopes, it appears that the antibodies bind to material in the rings, therefore it is quite feasible that this binding does not encroach upon the central channel.

A number of mechanisms could account for the inhibition of nuclear protein import by RL1. Since the antibody appears to slow the rate of transport rather than causing a total block, it is plausible that it binds to receptors, decreasing the number available for interaction with nuclear proteins, or slows the translocation step by preventing interaction with components active in subsequent steps of transport. Alternatively, RL1 may have a steric blocking effect on the gated channel or on other structural changes that may be required for mediated transport. The antibody could also inhibit mediated transport by cross-linking structural components of the pore such that protein conformational changes are blocked. This can be tested using  $F_{ab}$  fragments, however, so far we have been unable to prepare  $F_{ab}$  fragments from RL1 that retain their high-affinity binding on immunoblots.

Wheat germ agglutinin (WGA), a lectin that binds to *N*-acetyl glucosamine and sialic acids, also blocks protein import into nuclei in vitro (22) and in vivo (8, 54). WGA binds to sites on the nuclear pore complex as well as to numerous other sites within the nucleus and in the cytoplasm (10, 22, 29, 48). Although it is possible that inhibition by WGA is due to binding to the pore complex, indirect inhibition of nuclear protein import due to binding to other sites in the nucleus and cytoplasm is also conceivable.

### RNA Export Inhibition

RL1 also inhibits RNA export from the nucleus. This inhibition occurs when the antibody is injected either into the nucleus or cytoplasm. Since the RL1 antigens are known to be distributed on both faces of the pore (49), this is not surprising. Inhibition is most effective from the nucleoplasmic side of the envelope. This could be accounted for by the fact that the antibody is more concentrated in the nucleus than in the

cytoplasm; some antibody may be deposited into the cytoplasm during the nuclear injections, so compounding the inhibitory effect, or RL1 may interact with different components on the nucleoplasmic face, resulting in more marked inhibition. In rat liver nuclear envelopes one epitope, found only on the 180,000-*M<sub>r</sub>* polypeptide, is disposed only on the nucleoplasmic face of the pore complex (49). It is possible that all RL1 antigens are asymmetrically distributed between the nuclear and cytoplasmic sides of the envelope, therefore, one might expect different effects when the antibodies are applied to different faces of the pore.

Transport of tRNA and 5S RNA was differentially inhibited by RL1. 5S RNA export was inhibited more than that of tRNA. This may reflect packaging of RNA into RNP particles. When 5S RNA is injected into oocytes it is transported to nucleoli (12), the site of ribosome assembly, where it may be packaged into large ribosomal subunits before export to the cytoplasm. It is thus possible that newly synthesized tRNA is exported more rapidly than 5S RNP particles and the observed inhibition of transport may, therefore, be less marked for tRNA.

In summary, we have demonstrated that an antibody against the nuclear pore complex specifically inhibits mediated transport in both directions across the nuclear envelope. This inhibition seems to be an effect on the mediated functions of the pore complex but not on the ability to support diffusion of small proteins. The antibody thus distinguishes between these two properties of the pore complex. This provides a good basis from which to pursue the role of these unusual glycoproteins in the function of the pore complex.

We would like to thank Tom Urqhart for photographic work, and Alayne Senior and Claudette Snow for technical assistance. The manuscript has benefitted from the criticisms of Stephen Adam, James Glass, Urs Greber, and Ron Milligan.

This work was supported by an American Cancer Society (MD division) starter grant to C. Featherstone, a U. K. Medical Research Council traveling fellowship to M. K. Darby and grants from the American Cancer Society and National Institutes of Health to L. Gerace.

Received for publication 20 May 1988, and in revised form 18 July 1988.

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